

What is claimed is:

- 1        1. A method for determining whether a test compound is an inhibitor of bacterial  
2        tetrahydrofolate biosynthesis, the method comprising:  
3            (i) contacting a bacterial cell with a test compound, wherein the bacterial cell  
4        contains a promoter, the activity of which is increased in the presence of a compound that  
5        inhibits tetrahydrofolate biosynthesis; and  
6            (ii) measuring activity of the promoter, wherein an increase in activity, relative to  
7        the level of activity of the promoter in the absence of the test compound, indicates that the  
8        test compound is an inhibitor of bacterial tetrahydrofolate biosynthesis.
- 1        2. A method of claim 1, wherein the promoter is *panB*.
- 1        3. A method of claim 2, wherein the *panB* promoter comprises a nucleic acid  
2        sequence as set forth in SEQ ID NO:1 or a fragment thereof.
- 1        4. A method of claim 1, wherein the activity of the promoter is measured by an  
2        antibody specific for a polypeptide selected from the group consisting of: aspartate 1-  
3        decarboxylase, pantothenate synthase, and ketopantoate hydroxymethyltransferase.
- 1        5. A method of claim 1, wherein the activity of the promoter is measured by an  
2        assay for the activity of an enzyme selected from the group consisting of: aspartate 1-  
3        decarboxylase, pantothenate synthase, and ketopantoate hydroxymethyltransferase.
- 1        6. A method of claim 1, wherein the activity of the promoter is measured by  
2        detecting the RNA species transcribed from the gene regulated by the promoter.
- 1        7. A method of claim 1, wherein the cell contains the promoter operably linked to a  
2        reporter gene.

1 8. A method of claim 7, wherein the reporter gene is selected from the group  
2 consisting of *lacZ*, *cat*, *gus*, a luciferase gene, and a green fluorescent protein gene.

1 9. A method for determining whether a test compound is an inhibitor of bacterial  
2 tetrahydrofolate biosynthesis, the method comprising:

3 (i) contacting a bacterial cell with a test compound, wherein the bacterial cell  
4 contains

5 (a) a promoter, the activity of which is increased in the presence of a compound  
6 that inhibits tetrahydrofolate biosynthesis, operably linked to

7 (b) a reporter gene; and

8 (ii) measuring activity of the promoter, wherein an increase in activity, relative to the  
9 level of activity of the promoter in the absence of the test compound, indicates that the test  
10 compound is an inhibitor of bacterial tetrahydrofolate biosynthesis.

1 10. A method of claim 9, wherein the reporter gene is selected from the group  
2 consisting of *lacZ*, *cat*, *gus*, a luciferase gene, and a green fluorescent protein gene.

1 11. A method of claim 9, wherein measuring an increase in activity of the promoter  
2 comprises measuring binding of antibodies to a product of the reporter gene.

1 12. A method of claim 9, wherein the promoter is *panB*.

1 13. A method of claim 12, wherein the *panB* promoter comprises a nucleic acid  
2 sequence as set forth in SEQ ID NO:1 or a fragment thereof.

1 14. A method for determining whether a test compound is an antibacterial agent, the  
2 method comprising:

3 (i) contacting a bacterial cell with a test compound, wherein the bacterial cell  
4 contains

5 (a) a promoter, the activity of which is increased in the presence of a compound  
6 that inhibits tetrahydrofolate biosynthesis, operably linked to

7 (b) a reporter gene;

(ii) measuring activity of the promoter, wherein an increase in activity, relative to the level of activity of the promoter in the absence of the test compound, indicates that the test compound is an inhibitor of bacterial tetrahydrofolate biosynthesis; and

(iii) determining whether the inhibitor of tetrahydrofolate biosynthesis inhibits the growth of a bacterium, wherein a compound that inhibits the growth of a bacterium is an antibacterial agent.

15. A method of claim 14, further comprising assaying the test compound for its ability to inhibit tetrahydrofolate biosynthesis.

16. A method of claim 15, wherein inhibition of tetrahydrofolate biosynthesis is detected as inhibition of *para*-aminobenzoic acid (PABA) uptake into cells.

17. A method of claim 15, wherein inhibition is measured in a biochemical assay with a cell extract for an enzyme activity which is required for tetrahydrofolate biosynthesis.

18. A method of claim 17 wherein the enzyme activity assayed is selected from the group consisting of: GTP cyclohydrolase, 7,8 dihydroneopterin aldolase, 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, dihydropteroate synthase, aminodeoxychorismate synthase, aminodeoxychorismate lyase, dihydrofolate:folyl-polyglutamate synthase, and dihydrofolate reductase.

19. A method of claim 14, wherein the promoter is *panB*.

20. A method for treating a bacterial infection in an organism, the method comprising administering to the organism an antibacterial agent identified by the method of claim 14, in an amount effective to treat the bacterial infection.

21. A method of claim 20, wherein the organism is a mammal.

22. A method of claim 21, wherein the mammal is a human.

23. A method of claim 20, wherein the bacterial infection is caused by *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus endocarditis*, *Streptococcus faecium*, *Streptococcus sanguis*, *Streptococcus viridans*, or *Streptococcus hemolyticus*.

24. A method of claim 20, wherein the bacterial infection is an infection by a pathogenic bacterium.

25. A method of claim 20, wherein the bacterial infection is an infection by a non-pathogenic bacterium.

26. A composition comprising an antibacterial agent identified by the method of claim 14 and a pharmaceutically acceptable carrier.

27. A method of preparing an inhibitor of bacterial tetrahydrofolate biosynthesis, the method comprising:  
screening multiple test compounds by the method of claim 9;  
identifying candidate compounds that increase promoter activity;  
identifying, and selecting from the candidate compounds a lead compound that inhibits bacterial tetrahydrofolate biosynthesis; and  
formulating the selected lead compound as an inhibitor of bacterial tetrahydrofolate biosynthesis.

28. A method of preparing an antibacterial agent, the method comprising:  
screening multiple test compounds by the method of claim 14;  
identifying candidate compounds that upregulate promoter activity;  
identifying and selecting from the candidate compounds a lead compound that inhibits growth of a bacterium; and  
formulating the selected lead compound as an antibacterial agent.

1        29.    A method for preparing an inhibitor of bacterial tetrahydrofolate biosynthesis, the  
2 method comprising:

3                screening multiple test compounds by the method of claim 9;  
4                identifying candidate compounds that increase promoter activity;  
5                isolating a lead compound from the candidate compounds;  
6                derivatizing the isolated lead compound, to produce derivatives of the lead  
7 compound;  
8                identifying a derivative that inhibits bacterial tetrahydrofolate biosynthesis; and  
9                formulating the identified derivative as an inhibitor of bacterial tetrahydrofolate  
10 biosynthesis.

1        30.    An inhibitor of bacterial tetrahydrofolate biosynthesis prepared by the method of  
2 claim 29.

1        31.    A method for inhibiting bacterial tetrahydrofolate biosynthesis in bacteria  
2 infecting an organism, the method comprising administering to the organism an antibacterial  
3 agent identified by the method of claim 30, in an amount effective to inhibit bacterial  
4 tetrahydrofolate biosynthesis in the bacteria.

1        32.    A method for preparing an antibacterial agent, the method comprising:  
2                screening multiple test compounds by the method of claim 14;  
3                identifying candidate compounds that increase promoter activity;  
4                identifying and selecting a lead compound that inhibits growth of a bacterium  
5 from the candidate compounds;  
6                derivatizing the selected lead compound to produce derivatives of said lead  
7 compound;  
8                identifying a derivative that inhibits growth of a bacterium; and  
9                formulating the identified derivative as an antibacterial agent.

1        33.    An antibacterial agent prepared by the method of claim 32.

1        34.     A composition comprising an antibacterial agent of claim 33 and a  
2     pharmaceutically acceptable carrier.

1        35.     A method for inhibiting growth of bacteria in an organism having a bacterial  
2     infection, the method comprising administering to the organism a composition of claim 34, in  
3     an amount effective to inhibit growth of bacteria in the organism

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